INSTRUCTIONS FOR USE

CAMPYLOBACTER AGAR BLASER WANG

Ready-to-use plates

Campylobacter jejuni
on Campylobacter Agar Blaser Wang

1 - INTENDED USE

In vitro diagnostic device. Selective medium for the isolation of *Campylobacter* spp. from faecal specimens.

2 - COMPOSITION - TYPICAL FORMULA*

Peptocomplex	10.0 g
Tryptose	10.0 g
Peptone	3.0 g
Maize starch	1.0 g
Sodium chloride	5.0 g
Agar	12.0 g
Amphotericin B	2.0 mg
Cephalothin	15.0 mg
Trimethoprim	5.0 mg
Vancomycin	10.0 mg
Polymyxin B	2500 IU
Defibrinated sheep blood	50 mL
Purified water	950 mL

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Campylobacter spp. are Gram-negative, oxidase-positive, non-sporeforming, curved, spiral, or S-shaped rods, 0.2–0.9 µm wide and 0.5–5 µm long. Organisms are usually motile by means of a single polar unsheathed flagellum at one or both ends, that gives them a very characteristic “corkscrew” motility.[1] They are nutritionally fastidious and grow under strictly anaerobic or microaerobic (containing approximately 5-10% O_2 and 5-10% CO_2 for recovery) conditions, but a number of *Campylobacter* species, including *C.concisus, C.curvus, C.gracilis, C.mucosalis, C.rectus, C.showae* and some strains of *C.hyointestinalis,* require a hydrogen-enriched atmosphere (3-7% H_2 is required) for growth, a condition not routinely used in the diagnostic laboratories.[2]

Gastrointestinal *Campylobacter* infections are acquired by ingestion of undercooked poultry, seafood, meat and produce, by the contact with animals and by drinking untreated water or milk. In some instances, infection can progress to life-threatening extra-gastrointestinal diseases.[2]

C.jejuni accounts for about 90% of reported infections and most of the remainder are caused by *C.coli* and *C.lari*; other *Campylobacter* species have also been isolated from cases of diarrhoea (*C.helveticus, C.upsaliensis, C.hominis, C.gracilis, C.lanienae, C.peloridis, C.concisus, C.mucosalis, C.fetus, C.hyointestinalis, C.sputorum , C.insulaenigrae*).[2] The species most commonly associated with disease in humans are thermophilic, i.e. they will grow at 42-43°C and 37°C, but not at 25°C; *C.jejuni* subspecies *doyley*, *C.fetus*, and C.*fetus* subspecies *venerealis* do not grow at 42°C.[3]

Since the early 1970s, when *C.jejuni* and *C.coli* have been recognised as agents of gastrointestinal infections associated with food poisoning, several liquid and plated culture media have been developed, originally designed for the examination of faeces and then extended to the detection of *Campylobacter* in food and water.[3] The selective media for isolation of *Campylobacter* consist of a non-selective base to be used with or without animal blood and of a mixture of antimicrobial compounds; among the isolation media proposed in the literature, the review by Corry and Atabay[3] mentions the following media: Skirrow, Blaser Wang, Preston, mCCD Bolton, mCCD Hutchinson and Bolton, Karmali, Line TTC.

Campylobacter Agar Blaser Wang (known also as Campy-BAP) is prepared according to the formulation devised by Blaser and Wang,[4] who modified the formulation of Skirrow by adding cephalothin and amphotericin B and substituting laked horse blood with defibrinated sheep blood. The peptones supply nitrogen, carbon, and trace elements for microbial growth. Yeast extract is a source of the group B vitamins. Sheep blood supplies additional nutrients. The selective agents of the medium are vancomycin, with a strong inhibitory activity against Gram-positive bacteria, polymyxin B, cephalothin and trimethoprim, which mainly suppress the growth of Gram-negative bacteria and amphotericin B, included as an antifungal compound.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Campylobacter Agar Blaser Wang	Ready-to-use plates	541111	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, controlled atmosphere generators and jars, ancillary culture media and reagents for the identification of the colonies.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@biolifeitaliana.it; export@biolifeitaliana.it; web: www.biolifeitaliana.it

7 - SPECIMENS

Faecal specimens are preferred for isolating *Campylobacter* spp. from patients with gastrointestinal infections; however, rectal swabs are acceptable for culture.[5] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.

8 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Solid faeces: faeces may be diluted 1:4 in sterile saline solution or 0.1% peptone water. It has been shown that dilution significantly reduces the amount of competing flora without compromising isolation of low numbers of pathogens.[3] Inoculate 3-5 drops on the medium surface.
Liquid stool: inoculate 3 drops on the medium surface.
Rectal swabs: roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
For all type of specimens, streak with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.
Incubate in a microaerobic atmosphere consisting approximately of 5% O_2, 10% CO_2, and 85% N_2, at 39-42°C for 40-48 hours.[3,]
If non-thermophilic species should be isolated, incubate inoculated plates at 37 ± 2°C in a microaerobic atmosphere.

9 - READING AND INTERPRETATION

After incubation observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Campylobacter colonies are usually grey/white or creamy grey in colour, swarming and moist in appearance. They may appear as a layer of growth over the surface of the agar. Colonies are usually non-pigmented.
Campylobacter species are oxidase positive. If a colony phenotypically resembling *Campylobacte*r species is oxidase negative, subculture to blood agar and retest after 24hr incubation.[2]
The presumptive identification of thermophilic and enteropathogenic *Campylobacte*r can be done on the basis oxidase test (+) and the characteristic motility. For a complete explanation of the identification criteria and methods, refer to the quoted references.[2,5]

10 – USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[6]

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
C.jejuni	ATCC 33291	41-42°C / 40-48h / M	good growth
E.coli	ATCC 25922	41-42°C / 40-48h / M	partially or totally inhibited

M: microaerobic incubation; ATCC is a trademark of American Type Culture Collection

11 – PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all batches of ready-to use plates of Campylobacter Agar Blaser Wang and of the raw materials used for the production of prepared plates (dehydrated Columbia Agar Base, REF 401136 supplemented with Blaser Wang Antimicrobic Supplement and defibrinated sheep blood), are tested for productivity and selectivity by comparing the results with previously approved Reference Batches.
Productivity is tested by a semi-quantitative ecometric technique with the target strains *C.jejuni* ATCC 33291 and *C.coli* ATCC 43478. After incubation at 41-42°C for 40-48h hours in microaerobic incubation the amount of growth is evaluated and recorded. Both strains show a good growth.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *C.albicans* ATCC 60193, *E.coli* ATCC 25922, *S.aureus* ATCC 25923, *E.faecalis* ATCC 19433, *P.aeruginosa* ATCC 27853, *P.rettgeri* ATTC 39944, *P.mirabilis* ATCC 12453. The growth of *C.albicans*, *E.coli, P.aeruginosa* and *P.rettgeri* is partially inhibited, while the other non-target strains are totally inhibited.

12 – LIMITATIONS OF THE METHOD

- Cephalothin and polymyxin B can be inhibitory to some strains of *C.jejuni* and *C.coli* and also to many of the other less commonly encountered *Campylobacter* species, such as C.upsaliensis, C.hyointestinalis, and *C.fetus*.[2]
- To achieve the highest yield of *Campylobacter* from stool samples, a combination of media based on different selective systems appears to be the optimal method (e.g., Blaser Wang medium and a less selective Campylobacter blood free selective medium, such as Karmali or CCDA).[2]
- Some species of *Campylobacter*, such as *C.concisus, C.rectus, C.curvus, C.gracilis* and *C.showae* require increased hydrogen for primary isolation and growth. These species are usually not recovered under the conventional microaerobic conditions with the hydrogen concentration lower than 2%.[2,5]
- *Campylobacter* species have different optimal temperature for growth. The choice of incubation temperature for routine cultures of stool is critical in determining the spectrum of species that will be isolated.[5] *Campylobacter jejuni* subspecies *doylei*, *Campylobacter fetus* and *C.fetus* subspecies *venerealis* do not grow at 42°C.[3]
- Blood free formulations (e.g., Karmali, CCDA) appear to have better performances than blood containing media.[5]
- The clinical advantage of enrichment broths formulated to enhance the recovery of *Campylobacter* has not been studied adequately.[5] Enrichment seems not to be necessary for samples collected in the acute campylobacteriosis phase, while *Campylobacter* recovery increases in asymptomatic patients, in studies involving low numbers of the target organism, in samples not readily sent to the laboratory and in samples taken in the convalescence phase after an episode of diarrhoea.[7,8]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- The culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 – PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the ready-to use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 – STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 – REFERENCES

1. Corry JEL, Atabay HI. Culture Media for the Isolation of Campylobacters, Helicobacters and Arcobacters. *In* Handbook of Culture Media for Food and Water Microbiology, Edited by Corry JEL, Curtis GDW, Baird RM. Published by the Royal Society of Chemistry, 3rd Edition 2012.
2. Public Health England. Identification of Campylobacter species. ID23. Issue 3.1. 2018.
3. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. ID30. Issue 8.1. 2014.
4. Blaser MJ, Berkowicz ID, Laforce FM, Cravens J, Reller LB, Wang WL. Campylobacter enteritis: clinical and epidemiological features. Ann Intern Med 1979; 91:179–185.
5. Nachamkin I. Campylobacter and Arcobacter. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
6. CLSI (formerly NCCLS). Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
7. Bolton FJ, Robertson L. A selective medium for isolating Campylobacter jejuni/coli. J Clin Pathol 1982; 35:462.
8. Hutchinson DN, Bolton FJ. Is enrichment culture necessary for the isolation of Campylobacter jejuni from faeces? J Clin Pathol 1983; 36:1350-1352.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/09
Revision 2	Removal of obsolete classification	2023/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
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